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SEROTYPING AND ANTIMICROBIAL DRUG RESISTANCE OF *SALMONELLA* ISOLATED FROM LETTUCE AND HUMAN DIARRHEA SAMPLES IN BURKINA FASO.

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Abstract

Background: In Burkina Faso dirty water in particular those of the stoppings and the gutter ones are used for vegetables irrigation in the gardens. The aim of this study was to determine the prevalence and antibiotic susceptibility of *Salmonella* serotypes from humans and lettuce samples in Burkina Faso.

Materials and Methods: *Salmonella* strains isolated from patients in 2009 to 2015 and lettuce samples in 2014 in Burkina Faso were serotyped using specific antisera. All strains were subjected to a set of 14 antibiotics to study their antibiogram by using Baeur–Kirby disk diffusion method.

Results: Out of 154 *Salmonella* isolated, 60 were from human and 94 from lettuce samples. Serotyping revealed four different serotypes and 39% (60) untypeable strains from human and lettuce (14 and 46 strains). *Salmonella* serotypes from human and lettuce samples were: Paratyphi A (10% and 22%), Paratyphi B (34% and 8%), Paratyphi C (14% and 18%) and Typhi (21% and 1%). A high resistance of *Salmonella* Paratyphi B and *Salmonella* spp to tetracycline were 70% from human and 35 % from lettuce samples. Multiresistance was observed to tetracycline, chloramphenicol and amoxicillin/clavulanic-acid or ampicillin with *Salmonella* Paratyphi B 35% and *Salmonella* Typhi 33% from human samples and *Salmonella* spp 4% from lettuce samples.

Conclusion: This study showed the diversity of *Salmonella* serotypes from both clinical and environmental samples and emergence of multiresistant *Salmonella* to antibiotics in Burkina Faso. A lettuce is a potential source of transmission of *Salmonella* causing diarrhea among human in Burkina Faso.

Keywords: *Salmonella* serotypes, antimicrobial susceptibility, lettuce, human

List of non-standard Abbreviations : HDB: Hôpital du District de Bogodogo, LNSP: Laboratoire National de Santé Publique,

DSG : District Sanitaire de Gourcy, DSB : District Sanitaire de Boromo

Introduction

Salmonella is an enteric bacterial pathogen and a major pathogenic bacterium that causes food poisoning. Its routes of infection include contaminated foods and water. *Salmonella* species are leading causes of acute gastroenteritis in several countries and salmonellosis remains an important public health problem worldwide, particularly in the developing countries (Rotimi et al., 2008). Developing countries are more concerned by a broad range of these diseases among which appears cholera, campylobacteriosis, infections with *Escherichia coli*, shigellosis, brucellosis, hepatitis A and salmonellosis. In this numerous of foodborne infections, salmonellosis is the most frequent infection with a great number of serotypes and intoxications caused with lethality in 1% cases (Ao et al., 2015; Assi-Claire, 2000). Among the most foodborne infections with *Salmonella*, the lettuce takes up a significant place.

In Burkina Faso, rains shortage leads to the practice of the farming irrigated by barrage or waste water. It is the case of the truck farmer production. The dirty water in particular those of the stoppings and the gutter ones are used for the vegetables irrigation. These vegetables mainly lettuce are generally contaminated by the enteric bacteria in particular *Salmonella* from this contaminated water (Traoré et al., 2015). According to Petterson et al. (2010), the consumption of

the fruit and vegetables constitutes a factor of potential risk of infection by bacteria enteropathogens such as *Salmonella* and *Escherichia coli* O157. Cases of food poisoning related to the contaminated vegetable ingestion were identified a little everywhere in the world (Wendel et al., 2009). Among the factors generally implicated in the contamination of vegetables appears the irrigation water (Koffi-Nevry et al., 2011).

Drug resistance among *Salmonella* strains has emerged worldwide, making antimicrobial susceptibility testing an important role in public health laboratories. Antibacterial agents are often recommended for the treatment of suspected salmonellosis. Patients were not responding to the most available antibiotics of choice. Those practices can enhance the antibiotics resistances genes. It is now generally accepted that the main risk factor for the increase of resistance to pathogenic bacteria is the anarchic use of antibiotics. Previous studies in Burkina Faso showed that *Salmonella enterica* isolated from meat and several foods is resistant to commonly used antibiotics like amoxicillin/clavulanic-acid, aztreonam, cefalotin, ceftriaxone, cefepim, gentamicin, chloramphenicol, tetracycline, nalidixic-acid and ciprofloxacin (Bagré et al., 2014; Bsadjo Tchamba et al., 2015).

In this study, we examined lettuce samples from garden and stools samples from diarrheic persons to determine *Salmonella*. Specifically, the aims of this study were (1) to determine the *Salmonella* serotypes and antimicrobial resistance of the obtained isolates and (2) to compare the serotypes and resistance profiles to those previously obtained from the lettuce and human.

Materials and methods

Study design, population and settings

A total of 94 *Salmonella* were isolated from 134 lettuce samples collected in 2014 in the surrounding environments of the dam number 3 of Ouagadougou and the university hospital Yalgagado Ouédraogo, the biggest hospital in Burkina Faso. In addition, 60 *Salmonella* were isolated from 765 (447 in Ouagadougou, 125 in Boromo and 193 in Gourcy) patients with diarrhea between 2009 and 2015 in three regions in Burkina Faso: -Ouagadougou (*Hopital du District de Bogodogo* (HDB) and *Laboratoire National de Santé Publique* (LNSP)), which is the capital city located in the center of Burkina Faso;

-Gourcy (District Sanitaire de Gourcy (DSG))

-and Boromo (District Sanitaire de Boromo (DSB)), which are rural areas located in northern and western parts of the country.

All samples, human and lettuce were analysed at National Public Health Laboratory (LNSP) in Ouagadougou for pathogens isolation and stored for further analysis at -30°C.

Microbiological analyses

The suspected *Salmonella* samples were placed on Mueller Hinton Agar (Himedia, India) and incubated at 37°C for 18–24h. The colonies were subjected to biochemical reactions using Enteric API 20E according to manufactures' instructions (BioMerieux, France) for further confirmation. Serotyping was done by slide agglutination using *Salmonella* polyvalent A, B, C, T, Vi antisera (Bio-Rad, France) according to the Kauffmann-White classification scheme (Popoff et al., 2004). All isolates were also tested for susceptibility to 14 different antimicrobial agents using the disk diffusion method on Mueller Hinton II agar (Bio-Rad France) following the European Committee on Antimicrobial Susceptibility Instructions (EUCAST) guidelines (EUCAST, 2013). *E. coli* ATCC 25922 and ATCC 35218 were used as a control. The antimicrobial disks (Himedia, India) used were nalidixic-acid (30µg), ciprofloxacin (5µg), ampicillin (10µg), amoxicillin (25µg) cefotaxime (30µg), imipenem (10µg), tetracycline (30µg), gentamicin (10µg), chloramphenicol (30µg), ceftriaxone (30µg), norfloxacin (10µg), ticarcillin (75µg), amoxicillin/clavulanic-acid (30µg) and trimethoprim/sulfamethoxazole (25µg). Inhibition diameters of the antibiotics were interpreted according to the EUCAST (EUCAST, 2013). The multiresistant is defined as the resistance to at least three different antibiotics family (Magiorakos et al., 2011). Extended-spectrum β -lactamases (ESBL) activity was carried out by using amoxicillin/clavulanic-acid against cefotaxime, ceftriaxone.

Results

Bacterial isolates from humans and lettuce

Out of 154 *Salmonella* isolated, 39 % (60) were from human and 61 % (94) from lettuce samples. Of 60 *Salmonella enterica* isolates from human, 27 % (16/60) of strains were from rural and 73 % (44/60) from urban clinical samples. This study showed high prevalence of *Salmonella* from male 57 % than female 43 %. Our results showed that 40 % of *Salmonella* were isolated from patients aged 12-23 months and 27 % of patients aged 0-11 months reported *Salmonella* (Table 1).

Table 1: *Salmonella* distribution by locality, age and sex

Age (months)		0 - 11	12 - 23	24 - 35	36 - 47	48 - 59
Males	HDB	2	7	2	0	1
	LNSP	2	5	1	1	2
	DSB	6	1	1	0	0
	DSG	1	0	1	0	0
Sous-Total	33 (56,89%)	11	13	5	1	3
Females	HDB	2	4	4	2	1
	LNSP	1	3	1	0	1
	DSB	2	2	0	0	0
	DSG	0	2	0	0	0
Sous-Total	25 (43,11%)	5	11	5	2	2
Total		16 (27,58%)	24 (41,4%)	10 (17,2%)	3 (5,17%)	5 (8,62%)

Legend: %=percentage, HDB= Hopital de District de Bogodogo, LNSP= Laboratoire National de Santé Publique, DSB= District Sanitaire de Boromo, DSG= District Sanitaire de Gourcy.

Salmonella serotypes

Of 60 human *Salmonella* isolates, 77 % (46/60) were serotyped. Globally highest prevalence was observed to serotype Paratyphi B 34 % followed Typhi 20 %, Paratyphi C 13 % and Paratyphi A 10 %. According to the localities, the highest prevalence was observed with *Salmonella* serotype Paratyphi B: 3/4, 11/25 and 4/12 from DSG, HDB (Ouagadougou) and DSB respectively and then the serotype Typhi: 6/19, 1/4, 2/12 and 3/25 from LNSP, DSG, DSB and HDB respectively (Table 2). *Salmonella* spp were identified in 23 % (14/60). These isolates were not reacting to antisera used in our study.

Out of 94 *Salmonella enterica* isolates from lettuce, 51 % (48/94) reacted to the antisera used. The highest prevalence was observed to *Salmonella* serotype Paratyphi A 23 % followed by Paratyphi C 18 %, Paratyphi B 8 % and serotype Typhi 1 % (Table 3). *Salmonella* spp represented 49 % (46/94) of the strains did not react to the antisera used.

Antimicrobial susceptibility testing of human isolates

The *Salmonella* isolates originating from human were all resistant to fourteen (14) antibiotics. A higher frequency of antimicrobial resistance was observed to tetracycline 55 %, ticarcillin 38 %, amoxicillin/clavulanic-acid, ampicillin, amoxicillin 36 % and trimethoprim/sulfamexazol 33 %. Low frequency of resistance was observed to imipenem 3 %, ceftriaxon, cefotaxime 5 %, ciprofloxacin, norfloxacin, gentamicin 7 % and nalidixic-acid 10 % (Table 4). From different serotypes tested, the high resistance was observed with tetracycline 14/20, ampicillin, amoxicillin, amoxicillin/clavulanic-acid, ticarcillin 12/20, trimethoprim/sulfamexazol 11/20 and chloramphenicol 10/20 to *Salmonella* Paratyphi B. Resistance was observed to imipenem 02/20, ceftriaxone, cefotaxime, ciprofloxacin, norfloxacin, nalidixic-acid, and gentamicin 01/20 with *Salmonella* Paratyphi B. Extended-spectrum β -lactamases (ESBL) were not observed.

Table 2: *Salmonella* serotypes distribution by locality

Sites/Serotypes	S.Paratyphi A	S.Paratyphi B	S.Paratyphi C	S.Typhi	<i>Salmonella.spp</i>	TOTAL
HDB	01(04%)	11 (44%)	05 (20 %)	03(12 %)	05 (20 %)	25 (100%)
LNSP	03 (18%)	02 (12 %)	01 (06%)	06(35%)	05 (29 %)	17(100 %)
DSB	02 (17%)	04 (33%)	02 (17%)	02 (17%)	02 (17 %)	12 (100 %)
DSG	00	03 (75%)	00	01(25%)	00	04 (100 %)
TOTAL	06 (10%)	20 (34%)	08 (14%)	12(21 %)	12(21 %)	58 (100 %)

Legend: S= *Salmonella*, 00 = no prevalence, %=percentage, HDB= Hopital de District de Bogodogo, LNSP= Laboratoire National de Santé Publique, DSB= District Sanitaire de Boromo, DSG= District Sanitaire de Gourcy.

Table 3: *Salmonella* serotype from lettuce

Serotypes	<i>S.Paratyphi</i> A	<i>S.Paratyphi</i> B	<i>S.Paratyphi</i> C	<i>S.Typhi</i>	<i>Salmonella.spp</i>	Total
Prevalence	22 (23 %)	08 (09 %)	17 (18 %)	01 (01 %)	46 (49 %)	94(100 %)

Legend: *S*= *Salmonella*, %=percentage.+

Table 4: Frequency of antimicrobial resistance in *Salmonella* isolates from human

Antibiotiques	<i>Salmonella</i> serotypes					Total
	<i>S.Paratyphi</i> A N=06	<i>S.Paratyphi</i> B N=20	<i>S. Paratyphi</i> C N=08	<i>S. Typhi</i> N=12	<i>Salmonella.spp</i> N=12	
AMP	02(33%)	12(60%)	00	05(42%)	02(17%)	21(36 %)
AMX	02(33%)	12(60%)	00	05(42%)	02(17%)	21(36%)
AMC	02(33%)	12(60%)	00	05(42%)	02(17%)	21(36%)
CTR	00	01(05%)	00	00	02(17%)	3(5%)
IMI	00	02(10%)	00	00	00	2(3%)
CTX	00	01(05%)	00	00	02(17%)	3(5%)
TI	02(33%)	12(60%)	00	06(50%)	02(17%)	22(38%)
GEN	01(17%)	01(05%)	00	00	02(17%)	4(7%)
COT	02(33%)	11(55%)	00	04(33%)	02(17%)	19(33%)
NA	02(33%)	01(05%)	00	01(08%)	02(17%)	6(10%)
CIP	01(17%)	01(05%)	00	00	02(17%)	4(07%)
NX	01(17%)	01(05%)	00	00	02(17%)	4(07%)
C	01(17%)	10(50%)	00	05(42%)	01(08%)	17(29%)
TE	02(33%)	14(70%)	03(38%)	06(50%)	07(58%)	32(55%)

Legend: AMP= ampicillin, AMX= amoxicillin, AMC = amoxicillin/cluvulanic-acid, CRO = ceftriaxone, CTX= cefotaxime, NX= norfloxacin, COT= trimethoprim/sulfamexazol, C = chloramphenicol, CIP = ciprofloxacin, GEN = gentamicin, IMI = imipenem, NA = nalidixicacid, TE = tetracycline, TC = ticarcillin, % = percentage, *S* = *Salmonella*, N=number.

Antimicrobial susceptibility testing of lettuce isolates.

All 94 lettuce isolates were susceptible to imipenem, gentamicin, ceftriaxone, cefotaxime and ciprofloxacin. We observed a low antimicrobial resistance to tetracycline 22 %, amoxicillin/clavulanic-acid, amoxicillin, ampicillin, chloramphenicol, nalidixic-acid and trimethoprim/sulfamexazol respectively 7 %, 6 %, 5 %, 4 %, 3 %, and 2 %. The resistance was observed to tetracycline, amoxicillin/clavulanic-acid, amoxicillin, ampicillin, ticarcillin and trimethoprim/sulfamexazol, 01/17 with *Salmonella* Paratyphi C (Table 5).

Table 5: Frequency of antimicrobial resistance in *Salmonella* isolates from lettuce

Antibiotiques	<i>Salmonella</i> sérotypes					Total
	<i>S.Paratyphi</i> A N=22	<i>S. Paratyphi</i> B N=09	<i>S.Paratyphi</i> C N=17	<i>S.Typhi</i> N=01	<i>S.spp</i> N=46	
AMP	00	00	01(06%)	00	04(09 %)	5(05 %)
AMX	00	00	01(06%)	00	05(11 %)	6(06 %)
AMC	00	00	01(06 %)	00	06(13 %)	7(07 %)
CTR	00	00	00	00	00	00
IMI	00	00	00	00	00	00
CTX	00	00	00	00	00	00
TI	00	00	01(06%)	00	02(04%)	3(03%)
GEN	00	00	00	00	00	00
COT	00	00	01(06%)	00	01(02 %)	2(02 %)
NA	00	00	00	00	03(07 %)	3 (03 %)
CIP	00	00	00	00	00	00
NX	00	00	00	00	01(02%)	1(01%)
C	00	00	00	00	04(09 %)	4 (04 %)
TE	03(14%)	01(13 %)	01(06%)	00	16(35 %)	21(22 %)

Legend: AMP= ampicillin, AMX= amoxicillin, AMC = amoxicillin/cluvulanic-acid, CRO = ceftriaxone, CTX= cefotaxime, NX= norfloxacin, COT= trimethoprim/sulfamexazol, C = chloramphenicol, CIP = ciprofloxacin, GEN = gentamicin, IMI = imipenem, NA = nalidixic acid, TE = tetracycline, TC = ticarcillin, % = percentage, S = *Salmonella*, N=number

Multi-drugs resistance

This study revealed multidrugs resistance (resistance of three or more antibiotics with different families) with *Salmonella* Paratyphi B 07/20, *Salmonella* Typhi 04/12, *Salmonella* Paratyphi A 01/06 and *Salmonella* spp 02/12 from the clinical *Salmonella* isolates. However, *Salmonella* isolated from lettuce showed multidrugs resistance with *Salmonella* spp 02/46 (4 %). In the Hospital, multidrugs resistance observed with *Salmonella* Paratyphi B from DSB 03/04, DSG02/03, LNSP 01/02 and HDB 04/11 followed *Salmonella* Typhi from DSB, LNSP 03/06 and HDB 01/03.

Discussion

Salmonellosis is one of the major bacterial diseases transmitted from food. In this study, we investigated the distribution of *Salmonella* serotypes between human and lettuce isolates and highlight the consumption of lettuce as potential source of transmission of *Salmonella* causing diarrhea among human in Burkina Faso.

In Ouagadougou, people use generally waste water for vegetable irrigation. The high number of *Salmonella* strains isolated from lettuce was expected. Indeed, *Salmonella* is reported to be an environmentally persistent pathogen capable of surviving and proliferating in diverse environments (Winfield and Groisman, 2003).

In addition, our study site was a high risk of pathogens spread in environment. Vegetable farmers in our study locations depend largely on contaminated waste water from dam to irrigate their produce, while also using untreated animal manure as sources of nutrient supply to vegetables. Due to the vicinity, waste water from university hospital Yalgado Ouedraogo, the biggest hospital in the country is released into a canal which flows nearby the dam.

The distribution of serotypes of *Salmonella* from lettuce samples comprised *Salmonella* Typhi 1%, *Salmonella* Paratyphi 50% and *Salmonella* spp (untypeable) 49%. *Salmonella* Paratyphi has long been reported as a common cause of foodborne gastroenteritis (Hur et al., 2012). Also, the presence of *Salmonella* Typhi from lettuce is an indication of fecal contamination. Our finding supports the well-documented role of the presence of *Salmonella* in waste water and animals feces in environmental contamination in Burkina Faso as in some other developing countries (Kagambèga et al., 2013; Traoré et al., 2015).

The distribution of serotypes of *Salmonella* from human samples comprised *Salmonella* Typhi 21%, *Salmonella* Paratyphi 58% and *Salmonella* spp (untypeable) 21%. *Salmonella* Typhi was the second most common serotype isolated after *Salmonella* Paratyphi B. The high prevalence of *Salmonella* Typhi among isolates reveals that this pathogen still constitutes a significant public health importance in the country, therefore, appropriate control measures, such as vaccination and food safety measurements, need to be put in place. Recent study shown that *Salmonella* serotype Typhi and Paratyphi B were the most serotypes incriminated in diarrheal infections in Burkina Faso (Timbiné, 2014).

All serotypes Typhi, Paratyphi A, B and C were found in both human and lettuce samples. As reported in Burkina Faso and in the others parts of the world, *Salmonella* from fresh vegetables (carrots, radish, lettuce and parsley) play important role in foodborne disease (Afema et al., 2016; Islam et al., 2004; Koffi-Nevry et al., 2012; Traoré et al., 2015). We speculate that lettuce as well as fresh vegetables consumption without food safety measurement is a high risk of foodborne disease such as *Salmonella* different serotypes isolated but further research is needed to explore more this hypothesis by using modern techniques.

In addition, as the reservoir of typhoid and non-typhoid serotypes being water and animals and their products (OMS, 2013), shows that in Burkina Faso infections of *Salmonella* may be related to both sanitation and food hygiene.

The majority of the human isolates were resistant to tetracycline, ticarcillin, ampicillin, amoxicillin, and amoxicillin/cluvulanic-acid. Similar results have been reported in Burkina Faso and Nigeria (Bawa et al., 2016; Fashae et al., 2010). Multidrug resistance to these old and commonly used antimicrobials is a public health problem and could facilitate usage of fluoroquinolones and third-generation cephalosporins for empiric treatment (Yang et al., 2013). *Salmonella* Paratyphi B was the most resistant of the serotypes isolated from human isolates to urban and rural sites and exhibited resistance to tetracycline, ticarcillin, ampicillin, amoxicillin, amoxicillin/cluvulanic-acid and trimethoprim/sulfamexazol. Similar results have been reported in Burkina Faso (Bawa et al., 2016). In our study, it was noted the emergence of strains resistant to imipenem 10 %, ceftriaxone, cefotaxime, ciprofloxacin, norfloxacin, nalidixic-acid, and gentamicin (5 % to each antibiotic) with *Salmonella* Paratyphi B. This situation might be explained by the fact that in developing countries, populations are used to self-medication and use proscribed drugs sold on the street because of their low standard of living and education. These practices increase the phenomenon of bacteria resistance.

These observations call for regulation of antibiotics usage in Burkina Faso to avoid the spread of resistance to antimicrobials.

Our results showed that the great majority of *Salmonella* isolates from lettuce were more commonly susceptible to ciprofloxacin, ceftriaxone, cefotaxime, imipenem and gentamicin. However low resistances were observed to tetracyclin, ampicillin, amoxicillin, amoxicillin/clavulanic-acid and chloramphenicol. These results are consistent with those found in Burkina Faso in 2015 on isolated *Salmonella* strains in the environment (Traoré et al., 2015). A study on *Salmonella* isolated from animal feces in Ouagadougou was resistant to one or more tested antimicrobials (Kagambega et al., 2013). Others studies in Burkina Faso showed that *Salmonella enterica* isolated from meat and several foods is resistant to commonly used antibiotics like amoxicillin/clavulanic-acid, aztreonam, cefalotin, ceftriaxone, cefepim, gentamicin, chloramphenicol, tetracycline, nalidixic-acid and ciprofloxacin (Bagré et al., 2014; Bsadjo Tchamba et al., 2015).

Conclusion

To conclude, this study reports the presence of *Salmonella* similar serotypes in lettuce samples from irrigation sites and human isolates in Burkina Faso. The Consumption of lettuce without proper disinfection represents a serious public health risk. It also shows the emergence of multiresistant bacteria with clinical serotypes. The resistance of *Salmonella* serotypes to quinolones highlights the need for the establishment of a network and continuous monitoring of antibiotic resistance.

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Authors' contributions

SNS carried out the sampling and strains isolation, serotyping and their antibiotics susceptibility and drafted the manuscript, BOJ, OT, BIHN, TY, BN and SA supervised the sampling and strains isolation, serotyping, antibiotics susceptibility and participated in writing the manuscript. All authors read and approved the final version of the manuscript.

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